Cell Metabolism
Response

# Point Mutations Are Causing Progeroid Phenotypes in the mtDNA Mutator Mouse

***Editor's Note:*** *The December 2009 issue of* Cell Metabolism *included a Letter to the Editor from Vermulst and colleagues ("On Mitochondria, Mutations, and Methodology"). This Response to that Letter, from Edgar et al., was inadvertently omitted. We present here the Response, with our sincere apologies to all involved for the omission*.

The mtDNA mutator mice provide genetic evidence linking mtDNA mutations to aging phenotypes. These mice develop high levels of random point mutations within mtDNA (20–30 per genome) and contain ~25% linear deleted mtDNA molecules. We have demonstrated that the point mutations cause progressive respiratory chain deficiency, which, we propose, leads to premature aging (Edgar et al., 2009). This interpretation has been challenged by Loeb and coworkers, who argue that the two types of mtDNA mutations we found at very high levels in mtDNA mutator mice do not cause the phenotype. Instead, they argue that third type of mutation, circular deleted mtDNA molecules, are the culprit. We feel the published data from this group do not justify their conclusions, and we therefore performed the study recently published in *Cell Metabolism* (Edgar et al., 2009).

Loeb and coworkers reported a substantial increase of circular mtDNA molecules with deletions by using the random mutation capture (RMC) method. A recent study has given concerns about the performance of the RMC method (Greaves et al., 2009), and the results should therefore be interpreted with caution. We analyzed serial dilutions of DNA from mice that accumulate random multiple mtDNA deletions (Tyynismaa et al., 2005) and robustly detected deletions if present above 0.1% of total mtDNA in these control samples. In contrast, no such deletions were detected with the same assay in mtDNA mutator samples. Our results are supported by a recent study using an independent method; the single molecule PCR technique (Kraytsberg et al., 2009). We find it impossible to conclude that a very rare type of mtDNA mutation should override the importance of the two very abundant forms. Further support for our conclusion comes from the fact that mouse strains with substantially higher levels of single or multiple deleted circular mtDNAs show no features of premature aging (Tyynismaa et al., 2005).

Loeb and coworkers also mistakenly state that we report that "supercomplexes in the electron transport chain are unstable." We report no data on supercomplexes in our paper; however, we do report that the respiratory chain complexes are unstable, consistent with the conclusion that point mutations of mtDNA lead to the synthesis of respiratory chain subunits with amino acid substitutions that impair complex stability (Edgar et al., 2009). In summary, we feel the conclusions of our paper are well justified and that there is no convincing experimental evidence for a causative role for circular deleted mtDNA molecules in creating the progeroid syndrome of mtDNA mutator mice.

**Daniel Edgar,[1] Nils-Göran Larsson,[1,2] and Aleksandra Trifunovic[1,3,*]**
[1]Division of Metabolic Diseases, Department of Laboratory Medicine, Karolinska Institutet, S-14186 Stockholm, Sweden
[2]Max Planck Institute for Biology of Ageing, Gleueler Strasse 50a, D-50931 Cologne, Germany
[3]Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, D-50674 Cologne, Germany
*Correspondence: aleksandra.trifunovic@ki.se
DOI 10.1016/j.cmet.2009.12.001

## REFERENCES

Edgar, D., Shabalina, I., Camara, Y., Wredenberg, A., Calvaruso, M.A., Nijtmans, L., Nedergaard, J., Cannon, B., Larsson, N.G., and Trifunovic, A. (2009). Cell Metab. *10*, 131–138.

Greaves, L.C., Beadle, N.E., Taylor, G.A., Commane, D., Mathers, J.C., Khrapko, K., and Turnbull, D.M. (2009). Aging Cell *8*, 566–572.

Kraytsberg, Y., Simon, D.K., Turnbull, D.M., and Khrapko, K. (2009). Aging Cell *8*, 502–506.

Tyynismaa, H., Mjosund, K.P., Wanrooij, S., Lappalainen, I., Ylikallio, E., Jalanko, A., Spelbrink, J.N., Paetau, A., and Suomalainen, A. (2005). Proc. Natl. Acad. Sci. USA *102*, 17687–17692.